

Host-Dryinidae (Hymenoptera) interactions on edge grasses of maize agroecosystem throughout winter in Mexico

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Abstract

Little is known about the host-parasitoid interactions on the edges of crops during winter. Our objective was to determine the parasitoid species that attack nymphs and adults of leafhoppers and planthoppers, and evaluate the interactions that occur during winter on maize edges. Between January and June of 2014 and 2015 leafhoppers and planthoppers with evidence of parasitism by Dryinidae (Hymenoptera) were collected on grasses that grow in maize fields margins in Mexico and were maintained alive until parasitoid adult emergence. Dryinids such as *Gonatopus huggerti* (Olmi), *Gonatopus mimoides* (Perkins), *Gonatopus breviforceps* (Kieffer), and *Gonatopus caraibicus* (Olmi) were the most common parasitoids. They attacked several leafhopper species such as *Dalbulus maidis* (DeLong), *Balclutha incisa* (Matsumura), *Exitianus picatus* (Gibson), and *Stirellus bicolor* (Van Duzee). The connection of the net was of 31%. The presence of parasitoids in winter margins highlights the importance of conserving maize edges for biological control.

Keywords

Chrysidoidea, Auchenorrhyncha, Cicadellidae, biological control, Host-parasitoid interactions

Introduction

Edges in agroecosystems form a habitat hosting herbivore insects and their predators and parasitoids (Marshall and Moonen 2002, Ramsden et al. 2015). In addition, edges where perennial plants live, offer green resources during winter and become places with high insect biodiversity (Dennis and Fry 1992, Vollhardt et al. 2008). In addition, edges are important during winter when the crop is absent because they offer food, refuge, and sites for reproduction that help in the survival of herbivores and their natural enemies (Rabb et al. 1976, Marshall and Moonen 2002, Ramsden et al. 2015). Most of the edges in agroecosystems are formed by grasses (Marshall and Moonen 2002, Moya-Raygoza and Becerra-Chiron 2014), which contain a large number of herbivore leafhoppers (Hemiptera: Cicadellidae) (Hamilton and Whitcomb 2010).

Many leafhopper species are efficient vectors of viruses and bacteria to plants (Nault and Bradfute 1979, Weintraub and Beanland 2006). For example, in maize, which is one of the most important cereals in the world (Buckler et al. 2001), the leafhopper *Exitianus* sp. and *Exitianus obscurinervis* (Stål) (Hemiptera: Cicadellidae) transmit the bacterium Corn Stunt Spiroplasma (CSS) *Spiroplasma kunkelii* (Virla 2000, Carloni et al. 2011). Also, the leafhopper *Stirellus bicolor* (Van Duzee) (Hemiptera: Cicadellidae) transmits CSS, the Maize Rayado Fino Virus (MRFV), and the Maize Chlorotic Dwarf Virus (MCDV) (Nault and Bradfute 1979, Nault 1980, Wayadande and Nault 1993). Another vector is the corn leafhopper *Dalbulus maidis* (DeLong) (Hemiptera: Cicadellidae), considered the most important leafhopper pest of maize throughout Latin America, because it can transmit efficiently CSS, Maize Bushy Stunt Phytoplasma (MBSP), and MRFV (Nault 1990). In addition, leafhopper species that do not transmit plant pathogens inhabit edge grasses such as *Balclutha incisa* (Matsumura) (Hemiptera: Cicadellidae) (Narhardiyati and Bailey 2005) and *Amblysellus* sp. (Hemiptera: Cicadellidae). Leafhoppers that occur during the winter season on perennial grasses bordering maize fields are diverse with *S. bicolor*, *Graminella sonora* (Ball), *Dalbulus elimatus* (Ball), *D. maidis*, *B. incisa*, and *Exitianus picatus* (Gibson) (Hemiptera: Cicadellidae) being the most abundant species (Pinedo-Escatel and Moya-Raygoza 2015). However, none of the previous species were found to be positive for CSS or MBSP, suggesting that these two bacteria do not pass the winter in leafhoppers that overwinter on edge grasses (Torres-Moreno et al. 2015).

On the other hand, leafhoppers that inhabit edge grasses of maize fields were parasitized by dryinids and strepsipterans during the dry season (Moya-Raygoza and Becerra-Chiron 2014). Leafhoppers such as *Stirellus bicolor*, *G. sonora*, *E. picatus*, *Amblysellus* sp. and *D. maidis* are attacked by parasitoids. However, the species that attack these leafhoppers have not been investigated. Therefore, the objective of the present study was to search for adult parasitoids that attack nymphs and adult leafhoppers throughout the winter season on perennial grasses that border harvested maize agroecosystems and build a net between host species and parasitoid species to know the link among them.

Methods

The study was conducted in the region of Zapopan, state of Jalisco, Mexico 20°44'49"N, 103°30'48"W; 1,662 m above sea level. This region was selected because it represents the environmental condition in which maize is cultivated in most of the tropics. Maize is seeded there in June, when the wet season starts. Maize plants dry out in November and are harvested in December. From December to May grasses grow and have green foliage in the maize edges. This study was conducted during two consecutive winter seasons in 2014 and 2015, starting in January and ending in May. Sampling was done three times per week, using a sweep net for one hour between 11 am. and noon and performing 1,200 sweeps per sampling.

The collected hopper (leafhoppers or planthoppers) nymphs or adults with evidence of parasitism were maintained alive. Parasitized hoppers show on the thorax or abdomen a black sac hosting a larva of a parasitoid (Rios-Reyes and Moya-Raygoza 2004, Kathirithamby 2005). The live parasitized hoppers were transferred to leaf-cages on live grasses (*Brachiaria plantaginea* (Link) or *Digitaria ciliaris* (Retz) (Cyperales: Poaceae)), were grown in pots in a greenhouse. Each leaf-cage was 4.0 by 5.5 by 2.0 cm, with a small hole covered with fine mesh. Each hopper was maintained in a rearing room at 25 ± 2 °C, 50 % relative humidity, with a photoperiod of 12: 12 h (L:D) for a 45 days period. After 45 days each leaf-cage was reviewed to collect the emerged adult parasitoid and the hopper host, which were collected and stored in 70% ethanol for future identification. Parasitoids and hoppers were identified to genus or species level by different specialists in taxonomy. Voucher specimens were deposited in the entomological collection (CAJAPE) of the University of Guadalajara, Jalisco, Mexico and in the entomological collection (MOLC) of the University of Tuscia, Viterbo, Italy.

Network size, link density, dependence, and connectance were performed with Package bipartite (Dormann et al. 2008) of R software, and nestedness was conducted with ANINHADO (Guimarães and Guimarães 2006).

Results

The grasses found bordering the harvested maize fields during the 2014 and 2015 winter season were the following Cyperales-Poaceae: *Brachiaria plantaginea*, *Rhynchelytrum repens* (Willd), *Cynodon plectostachyus* (Schum.), *Sorgum vulgare* (Pers), and *Digitaria ciliaris*. They showed green foliage throughout the winter-dry season from January to May. Hoppers without and with parasitism evidence were found on these grasses.

Leafhopper (Hemiptera: Cicadellidae) nymphs and adults of the subfamily Deltocephalinae were parasitized mainly by wasps (Hymenoptera: Dryinidae) of the subfamily Gonatopodinae during two consecutive winter seasons of 2014 and 2015. Most of the leafhoppers with evidence of parasitism were collected in March, April, and May, when compared with those collected in January and February ($\chi^2 = 65.15$, $P < 0.05$).

Nymphs of *Exitianus* sp. and adults of *E. picatus* were parasitized in January and February by the following Dryinidae species: *Gonatopus huggerti* (Olmi), *Gonatopus mimoides* (Perkins) and *Gonatopus* sp. In addition, an undetermined species of Delphacidae was parasitized by an undetermined strepsipteran of the genus *Elenchus* (Strepsiptera: Elenchidae). In March, April, and May, more species of leafhoppers were parasitized. Nymphs and adults of *B. incisa*, *E. picatus*, *Amblysellus* sp., *D. maidis*, and *S. bicolor* were parasitized by the following Dryinidae: *G. huggerti*, *G. mimoides*, *Gonatopus breviforceps* (Kieffer), *Gonatopus caraibicus* (Olmi), and *Gonatopus* sp. (Table 1).

Dryinids of the genus *Gonatopus* attacked leafhoppers belonging to different tribes throughout the 2014 and 2015 winter seasons. For example, *G. mimoides* attacked leafhoppers of different tribes almost the whole winter season, parasitizing *D. maidis* and *B. incisa* (Macrostelini), *E. picatus* (Chiasmini), and *Amblysellus* sp. (Deltocephalini), whereas species such as *G. caraibicus* parasitized *Amblysellus* sp. (Deltocephalini) and *S. bicolor* (Stenometopiini) (Fig. 1).

Host (hopper)-parasitoid interactions are indicated by a qualitative network of species that occur on winter perennial grasses bordering harvested fields of maize. The network comprises 13 species (six parasitoid species and seven hopper host taxa) (Fig. 2). Every hopper species was parasitized by two species of parasitoids. *Gonatopus mimoides* and *G. huggerti* parasitize the largest proportion of the hopper species (four and three species respectively) while *G. breviforceps* parasitizes only one. Although *G. mimoides* was the species with the highest density of interactions, it shows a marked dependence (0.60) on *E. picatus*, in contrast with *G. huggerti* which showed three interactions, but with the same dependence (0.33) on each of them. The connectance was less than 0.31. The nestedness was 0.65; however, it was not significantly lower than expected by chance ($P > 0.05$).

Discussion

The present study is a report on the grass species (first trophic level), the herbivore hoppers and their nymphs (second trophic level), and adult parasitoids (third trophic level), that live during the winter-dry season on the edges of maize fields once the crop is harvested. Our results agree with those of Altieri and Letourneau (1982), Tscharrntke (2000), Marshall and Moonen (2002) and Ramsden et al. (2015), who found that edge vegetation is a key factor in the maintenance of parasitoids. Natural enemies abundance and diversity is increased by the vegetation on the edges in crops such as maize (Moya-Raygoza and Becerra-Chiron 2014), grapes (Nicholls et al. 2001, Altieri et al. 2005) and rice (Lou et al. 2013). In addition, edges not only function as habitat, but also as a site that provides hosts, food, and overwintering places for insect natural enemies (Sotherton 1985, Morris and Webb 1987).

Little is known about herbivore leafhopper vectors during winter season on the edges when the crop is absent. In maize, edge grasses host high diversity of leafhoppers, among which *Exitianus* sp. *B. incisa*, *S. bicolor*, *G. sonora*, *Amblysellus* sp., *E. picatus* and

Table 1. Adult parasitoids emerged (♀ or ♂) from the hopper in nymphal (N) or adult (A) stage collected on grasses during the winter seasons (January to May) of 2014 and 2015.

Parasitoid species	January	February	March	April	May
<i>Gonatopus huggeri</i> ♀	<i>Exitianus</i> sp. (N) <i>Exitianus picatus</i> (A)		<i>Balclutha incisa</i> (N)		
<i>Gonatopus mimoides</i> ♂ ♀		<i>Exitianus picatus</i> (A)	<i>Balclutha incisa</i> (A) <i>Exitianus picatus</i> (N)	<i>Exitianus picatus</i> (N) <i>Amblysellus</i> sp. (N) <i>Exitianus picatus</i> (A)	<i>Exitianus picatus</i> (A) <i>Exitianus picatus</i> (N) <i>Balclutha incisa</i> (A) <i>Dalbulus maidis</i> (A)
<i>Gonatopus breviforceps</i> ♀			<i>Balclutha incisa</i> (A)		<i>Balclutha incisa</i> (A)
<i>Gonatopus caraibicus</i> ♀				<i>Stirellus bicolor</i> (A) <i>Amblysellus</i> sp. (N) <i>Stirellus bicolor</i> (A)	<i>Stirellus bicolor</i> (A) <i>Amblysellus</i> sp. (A)
<i>Gonatopus</i> sp. ♂	<i>Exitianus</i> sp. (N)			<i>Amblysellus</i> sp. (N)	<i>Amblysellus</i> sp. (A)
<i>Elenchus</i> sp.	Planthopper (A)				
Total with evidence of parasitism	5	2	29	27	28

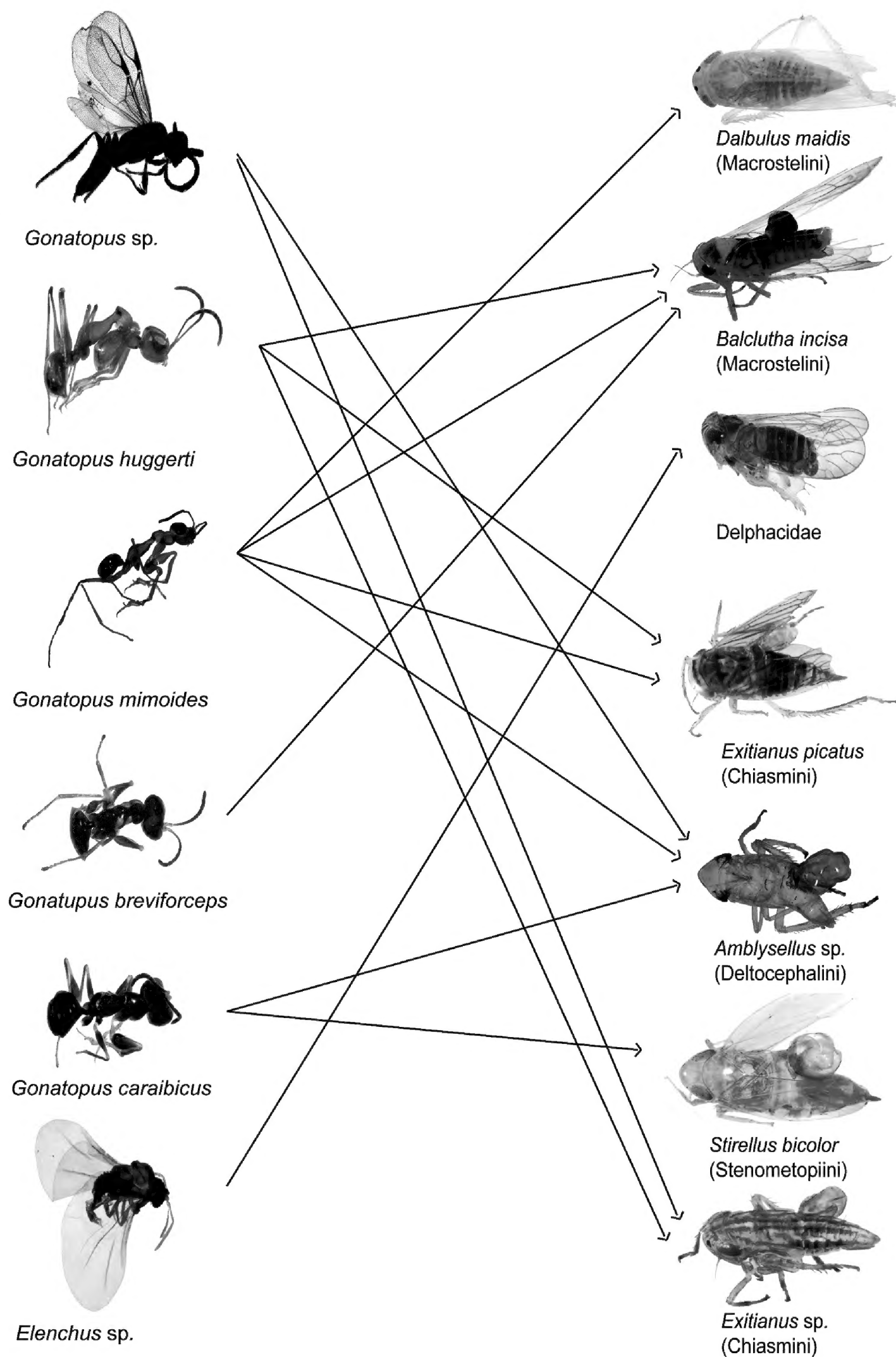


Figure 1. Total of parasitoid-host interactions found on the edges of maize agroecosystem in the winter seasons of 2014 and 2015. Name in parenthesis show the name of the host tribe.

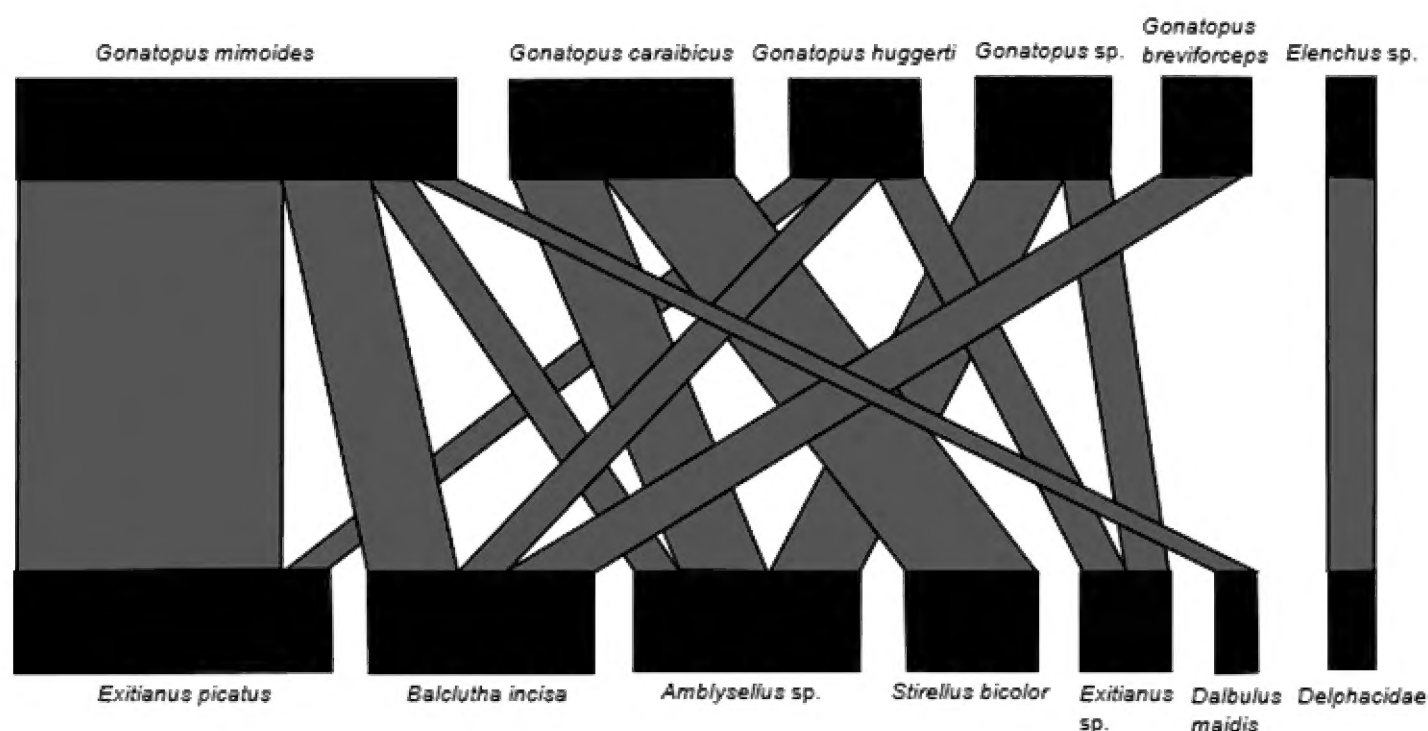


Figure 2. Quantitative food web of parasitoid-host interaction found on the edges of maize agroecosystem in the winter seasons of 2014 and 2015. Top names are the parasitoid species and bottom names are the host species.

D. elimatus are the most abundant species (Pinedo-Escatel and Moya-Raygoza 2015). Among the previous species, the literature indicates that the leafhopper *Exitianus* sp. transmits CSS (Virla 2000), *S. bicolor* transmits CSS, MRFV, and MCDV (Nault and Bradfute 1979, Nault and DeLong 1980), and *D. maidis* transmits CSS, MBSP, and MRFV (Nault 1990). Fortunately during winter edge grasses host leafhoppers that do not carry plant pathogenic bacteria and virus (Torres-Moreno et al. 2015). Results of the present study indicate grasses are green all the time bordering maize field and during the winter are a green food resource for leafhoppers free of plant pathogenic bacteria and viruses, but hosting nymph and adult parasitoids.

Six parasitoids species develop and emerge from nymph or adult hoppers. The most abundant leafhopper species were attacked by dryinids, among which most species are reported for first time on edge grasses. Previously, *G. caraibicus* was reported as a parasitoid of the leafhoppers *Amplipcephalus simpliciusculus* (Linnavuori), *Haldorus sexpunctatus* (Berg), *Chlorotettix* sp., *Planicephalus flavicosta* (Stål), *Exitianus obscurinervis* (Stål) and *Graminella stelliger* (Berg) (Guglielmino et al. 2013, Olmi and Virla 2014). In the present study *S. bicolor* and *Amblysellus* sp. leafhoppers are parasitized by *G. caraibicus* in April and May on edge grasses. For the first time *G. huggerti* is reported to attack *E. picatus* and *B. incisa* on grasses during winter. This parasitoid was found previously attacking *Exitianus* sp., *Polyamia tolteca* Kramer, *Polyamia satur* (Ball), *Planicephalus flavicosta* (Stål) and *Graminella comata* (Ball) in Mexico, Peru, Paraguay and Puerto Rico (Olmi et al. 2000, Garcete-Barret 2001, Moya-Raygoza and Olmi 2010, Olmi and Virla 2014). For the first time leafhopper hosts of *G. mimoides* are reported in Mexico: *E. picatus*, *B. incisa*, *D. maidis*, and *Amblysellus* sp. (for the USA see also Guglielmino et al. 2013). In addition, *G. breviforceps*, which has a Neo-

tropical distribution, is reported for the second time attacking *B. incisa* (see for the first time Moya-Raygoza and Olmi 2010). Most of the above new records were obtained because previous studies have not been focused on the edge grasses and the leafhopper-parasitoid interactions that occur on these winter grasses with green foliage.

Parasitoids attacked, developed and emerged from nymphs and adult leafhoppers belonging to different tribes. This report indicates that most parasitoids that live on the edge grasses during winter are generalists. Egg parasitoids, i.e. *Anagrus breviphragma* Soyka (Hymenoptera: Mymaridae), *Paracentrobia* sp. and *Pseudoligosita* sp. (Hymenoptera: Trichogrammatidae), also were generalists. They are the most common parasitoids attacking the eggs of *D. maidis* during the maize growing season in Mexico (Moya-Raygoza et al. 2012). These egg parasitoids attack not only the corn leafhopper but also the eggs of several species of cicadellids and delphacids (Moya-Raygoza et al. 2012).

Generalist parasitoids are important because a wide host range is a vital parameter with regard to a natural enemy's quality as a control agent (Salvo and Valladares 1997). Also generalist parasitoids show more plastic behavior and they have the ability to use a wide diversity of host resources, in which host availability changes through time (Raymond et al. 2015), particularly when abiotic factors such as temperature and humidity change during the winter-dry season on the maize edges. In central Mexico the lowest temperatures occur in January and February reaching 5 °C in January. Between March and May the lowest temperature was between 12 and 19 °C (Pinedo-Escatel and Moya-Raygoza 2015). Host-parasitoid food webs are altered by habitat modification in agriculture systems (Tylianakis et al. 2007) but no study has investigated the food web in field margins or edges of maize agroecosystems. This study shows that the food web on edges of maize fields supports the presence of herbivore leafhoppers and their parasitoids. The connection found in this study was less than 31%. Also the nesting index was of 0.65 indicating the network is not nested (Dunne et al. 2002).

Edges with reduced management intensity show an increase in immigration of insects, particularly natural enemies (Batáry et al. 2012). In our case, edges of maize fields are unmanaged, so they conserve the biodiversity of leafhoppers and their dryinid parasitoids, as found in the present study. A similar result was obtained by Moya-Raygoza and Becerra-Chiron (2014), concerning leafhopper egg parasitoids. This biodiversity occurs in part because grasses are green, resulting in food available for leafhoppers, which depend on a liquid diet. These grasses thus are a perennial habitat for leafhoppers and their parasitoids. Perennial edges also increase biodiversity (Altieri 1999, Werling et al. 2011). In addition, predation of pests in crops by different arthropods increases when perennial habitats such as grasslands and forests are found in the agricultural landscape (Dix et al. 1995, Werling et al. 2011). Conserving biodiversity on edges during winter is important for the natural enemies because they could colonize the new maize plants seeded in the wet season in June. Traditionally maize edges are considered sites for overwintering of insect pests (Free and Williams 1979) and local farms burn the edges and stubble. However burning the maize edges and stubble before seeding maize generates high level of carbon dioxide, affecting global warming (Li et al. 2007, Tylianakis et al. 2008).

In conclusion, edges that surround maize agroecosystem during winter hold leafhoppers from different tribes, which are attacked by generalist parasitoids. A food web of leafhoppers-parasitoids is presented for the first time for maize field edges formed by grasses. It is important to conserve this biodiversity of parasitoids for the conservation of natural biological control in maize edges.

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